CLAIMS

1. A variant of a parent Termamyl-like α-amylase, which variant has α-amylase activity, said variant comprises one or more mutations corresponding to the following mutations in the amino acid sequence shown in SEQ ID NO: 2:
T141, K142, F143, D144, F145, P146, G147, R148, G149,
Q174, R181, G182, D183, G184, K185, A186, W189, S193, N195
H107, K108, G109,D166, W167, D168, Q169, S170, R171, Q172, F173,
F267, W268, K269, N270, D271, L272, G273, A274, L275, K311, E346,
K385, G456, N457, K458,P459, G460, T461, V462, T463.

2. The variant according to claim 1, which variant has one or more of the following substitutions or deletions:
T141A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V;
K142A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
F143A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;
D144A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
F145A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;
P146A,D,R,N,C,E,Q,G,H,I,L,K,M,F,S,T,W,Y,V;
G147A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
R148A,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
G149A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
R181*,A,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
G182*,A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
D183*,A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
G184*,A,R,D,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
K185A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
A186D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
W189A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,Y,V;
S193A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,T,W,Y,V;
N195A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
H107A,D,R,N,C,E,Q,G,I,L,K,M,F,P,S,T,W,Y,V;
K108A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
G109A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
D166A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;

W167A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,Y,V;
D168A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
Q169A,D,R,N,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
S170A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,T,W,Y,V;
R171A,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
Q172A,D,R,N,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
F173A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;
Q174*,A,D,R,N,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
F267A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;
W268A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,Y,V;
K269A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
N270A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
D271A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
L272A,D,R,N,C,E,Q,G,H,I,K,M,F,P,S,T,W,Y,V;
G273A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
A274D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
L275A,D,R,N,C,E,Q,G,H,I,K,M,F,P,S,T,W,Y,V;
K311A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
E346A,D,R,N,C,Q,G,H,I,K,L,M,F,P,S,T,W,Y,V;
K385A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
G456A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
N457A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
K458A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
P459A,D,R,N,C,E,Q,G,H,I,L,K,M,F,S,T,W,Y,V;
G460A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
T461A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V;
V462A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y;
T463A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V.

3. The variant according to claim 2, wherein the variant has one or more of the following substitutions or deletions:
K142R; S193P; N195F; K269R,Q; N270Y,R,D; K311R; E346Q; K385R; K458R; P459T; T461P; Q174*; R181Q,N,S; G182T,S,N; D183*; G184*; K185A,R,D,C,E,Q,G,H,I,L,M,N,F,P,S,T,W,Y,V; A186T,S,N,I,V,R; W189T,S,N,Q.

4. The variant according to claims 1-3, wherein the variant has a deletion in position D183 + G184, and further one or more of the following substitutions or deletions: K142R; S193P; N195F; K269R,Q; N270Y,R,D; K311R; E346Q; K385R; K458R; P459T; T461P; Q174*; R181Q,N,S; G182T,S,N; D183*; G184*; K185A,R,D,C,E,Q,G,H,I,L,M,N,F,P,S,T,W,Y,V; A186T,S,N,I,V,R; W189T,S,N,Q.

5. The variant according to any of claims 1-4, wherein the variants exhibits an alteration in at least one of the following properties relative to the parent α-amylase:
i) improved pH stability at a pH from 8 to 10.5; and/or
ii) improved Ca^{2+} stability at pH 8 to 10.5, and/or
iii) increased specific activity at temperatures from 10 to 60°C, preferably 20-50°C, especially 30-40°C.

6. The variant according to any of claims 1-5, exhibiting improved stability at pH 8 to 10.5, having mutations in one or more of the position(s) corresponding to the following positions (using SEQ ID NO: 2 numbering): T141, K142, F143, D144, F145, P146, G147, R148, G149, R181, A186, S193, N195, K269, N270, K311, K458, P459, T461.

7. The variant according to claim 6, which variant has one or more of the following substitutions:
T141A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V;
K142A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
F143A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;
D144A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
F145A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;
P146A,D,R,N,C,E,Q,G,H,I,L,K,M,F,S,T,W,Y,V;
G147A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
R148A,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
G149A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
K181A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
A186D,R,N,C,E,Q,G,H,I,L,P,K,M,F,S,T,W,Y,V;

S193A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,T,W,Y,V;
N195A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
K269A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
N270A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
K311A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
K458A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
P459A,D,R,N,C,E,Q,G,H,I,L,K,M,F,S,T,W,Y,V;
T461A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V.

8. The variant according to claim 7, wherein the variant has one or more of the following substitutions: K142R, R181S, A186T, S193P, N195F, K269R, N270Y, K311R, K458R, P459T and T461P.

9. The variant according to claims 1-5, exhibiting improved Ca^{2+} stability at pH 8 to 10.5, having mutations in one or more of the following positions (using the SEQ ID NO: 2 numbering): R181, G182, D183, G184, K185, A186, W189, N195, N270, E346, K385, K458, P459.

10. The variant according to claim 9, which variant has one or more of the following substitutions or deletions:
R181*,A,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
G182*,A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
D183*,A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
G184*,A,R,D,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
K185A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
A186D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
W189A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,Y,V;
N195A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
N270A,R,D,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
E346A,R,D,N,C,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
K385A,R,D,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
K458A,R,D,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
P459A,R,D,N,C,E,Q,G,H,I,L,K,M,F,S,T,W,Y,V.

11.The variant according to claim 10, wherein the variant has one

or more of the following substitutions or deletions:
R181Q,N; G182T,S,N; D183*; G184*;
K185A,R,D,C,E,Q,G,H,I,L,M,N,F,P,S,T,W,Y,V; A186T,S,N,I,V;
W189T,S,N,Q; N195F; N270R,D; E346Q; K385R; K458R; P459T.

12. A variant according to claims 1-11, wherein the parent Termamyl-like α-amylase is selected from:

the *Bacillus* strain NCIB 12512 α-amylase having the sequence shown in SEQ ID NO: 1;

the *B. amyloliquefaciens* α-amylase having the sequence shown in SEQ ID NO: 5;

the *B. licheniformis* α-amylase having the sequence shown in SEQ ID NO: 4.

13. The variant according to claims 1-5, exhibiting increased specific activity at a temperatures from 10 to 60°C, preferably 20-50°C, especially 30-40°C, having mutation(s) in one or more of the following positions (using the SEQ ID NO: 2 numbering): H107, K108, G109, D166, W167, D168, Q169, S170, R171, Q172, F173, Q174, D183, G184, N195, F267, W268, K269,N270, D271, L272, G273, A274, L275, G456, N457, K458, P459, G460, T461, V462, T463.

14. The variant according to claim 13, which variant has one or more of the following substitutions:
H107A,D,R,N,C,E,Q,G,I,L,K,M,F,P,S,T,W,Y,V;
K108A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
G109A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
D166A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
W167A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,Y,V;
D168A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
Q169A,D,R,N,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
S170A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,T,W,Y,V;
R171A,D,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
Q172A,D,R,N,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
F173A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;

Q174*,A,D,R,N,C,E,G,H,I,L,K,M,F,P,S,T,W,Y,V;
D183*,A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V;
G184*,A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
N195A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
F267A,D,R,N,C,E,Q,G,H,I,L,K,M,P,S,T,W,Y,V;
W268A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,Y,V;
K269A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
N270A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
D271A,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
L272A,D,R,N,C,E,Q,G,H,I,K,M,F,P,S,T,W,Y,V;
G273A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
A274D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
L275A,D,R,N,C,E,Q,G,H,I,K,M,F,P,S,T,W,Y,V;
G456A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
N457A,D,R,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y,V;
K458A,D,R,N,C,E,Q,G,H,I,L,M,F,P,S,T,W,Y,V;
P459A,D,R,N,C,E,Q,G,H,I,L,K,M,F,S,T,W,Y,V;
G460A,D,R,N,C,E,Q,H,I,L,K,M,F,P,S,T,W,Y,V;
T461A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V;
V462A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,T,W,Y;
T463A,D,R,N,C,E,Q,G,H,I,L,K,M,F,P,S,W,Y,V.

15. The variant according to claim 14, wherein the variant has one or more of the following substitutions or deletions:
Q174*, D183*, G184*, N195F, K269S.

16. The variant according to claims 13-15, wherein the parent Termamyl-like α-amylase is the *B. licheniformis* α-amylase having the sequence shown in SEQ ID NO: 4.

17. A DNA construct comprising a DNA sequence encoding an α-amylase variant according to any one of claims 1-16.

18. A recombinant expression vector which carries a DNA construct according to claim 17.

19. A cell which is transformed with a DNA construct according to claim 17 or a vector according to claim 18.

20. A cell according to claim 19, which is a microorganism.

21. A cell according to claim 20, which is a bacterium or a fungus.

22. The cell according to claim 21, which is a Gram positive bacterium such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus lentus*, *Bacillus brevis*, *Bacillus stearothermophilus*, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus coagulans*, *Bacillus circulans*, *Bacillus lautus* or *Bacillus thuringiensis*.

23. Use of an α-amylase variant according to any one of claims 1-16 for washing and/or dishwashing.

24. A detergent additive comprising an α-amylase variant according to any one of claims 1-16, optionally in the form of a non-dusting granulate, stabilized liquid or protected enzyme.

25. A detergent additive according to claim 24 which contains 0.02-200 mg of enzyme protein/g of the additive.

26. A detergent additive according to claims 24 or 25, which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

27. A detergent composition comprising an α-amylase variant according to any of claims 1-16.

28. A detergent composition according to claim 27 which addi-

tionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

29. A manual or automatic dishwashing detergent composition comprising an α-amylase variant according to any one of claims 1-16.

30. A dishwashing detergent composition according to claim 29 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

31. A manual or automatic laundry washing composition comprising an α-amylase variant according to any one of claims 1-16.

32. A laundry washing composition according to claim 31, which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, an amylolytic enzyme and/or a cellulase.

33. Method for providing α-amylases with
1) altered pH optimum, and/or
2) altered temperature optimum, and/or
3) improved stability,
comprising the following steps:
i) identifying (a) target position(s) and/or region(s) for mutation of the α-amylase by comparing the molecular dynamics of two or more α-amylase's 3D structures having substantially different pH, temperature and/or stability profiles,
ii) substituting, adding and/or deleting one or more amino acids in the identified position(s) and/or region(s).

34. The method according to claim 33, wherein a medium temperature α-amylase is compared with a high temperature α-amylase.

35. The method according to claim 33, wherein a low temperature α-

amylase is compared with a medium or high temperature α-amylase.

36. The method according to claims 33-35, wherein the α-amylases are at least 70%, preferably 80%, up to 90%, such as up to 95%, especially 95% homologous.

37. The method according to claim 36, wherein the α-amylases compared are Termamyl-like α-amylases.

38. The method according to claim 28, wherein the α-amylases compared are any of the α-amylases shown in SEQ ID NO: 1 to SEQ ID NO: 8.

39. The method according to any of claims 33 to 38, wherein the stability profile of the α-amylases compared are the Ca^{2+} dependency profile.